REMARKS

Entry of the foregoing and favorable consideration of the subject application, in light of the following remarks, are respectfully requested.

By the present preliminary amendment, the specification has been amended to insert the attached paper copy of the Sequence Listing between the last page of the Disclosure (page 35) and the first page of the Claims. Further, the specification has been amended to insert the appropriate headings throughout the specification, and to insert the appropriate sequence listing identifiers throughout the specification. Additionally, in view of the formal drawings filed concurrently herewith, the specification has also been further amended to correct certain figure designations throughout the specification as well as in the Brief Description of the Drawings section of the specification.

Claims 1, 19 and 21 have been canceled without prejudice or disclaimer of the subject matter recited therein, and new claims 27-38 have been added. Support for new claims 27-28 and 29-30 can be found in prior claims 19 and 21, respectively. Support for new claims 31-32 and 33-34 can be found in originally filed claims 2 and 3, respectively. Support for new claim 35 can be found in originally filed claim 1, and support for new claims 36-38 can be found in originally filed claim 3 and on pages 22-23 of the specification. Accordingly, no new matter has been added.

In the event that there are any questions relating to this Preliminary Amendment, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that issuance of this application may be expedited.

Respectfully submitted,

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Date: April 19, 2001

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Page 1, Before Line 1 and After the Title

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. Application Serial No. 08/462,625 filed June 5, 1995.

Page 2, Paragraph Beginning at Line 37 to Page 3

These repetitive motifs (SEQ ID NO: 1) of 17 amino acids are represented by the formula:

Leu-Ala-Lys-Glu-Lys-Leu-Gln-X-Gln-Gln-Ser-Asp-Leu-Glu-Gln-Glu-Arg

in which X is Glu or Gly.

Page 3, Paragraph Beginning at Line 15 to Page 4

BRIEF DESCRIPTION OF THE DRAWINGS

Reference will be made in what follows to the Figures in which:

- Figure 1 (SEQ ID NO: 31) presents a recombinant protein of the invention of 316 amino acids, designated hereafter as antigen 536 or protein LSA-R-NR,
- Figure 2 (SEQ ID NO: 32) provides the nucleotide sequence of one of the recombinant nucleic acids studied (clone DG536) and which codes for the polypeptide LSA-R-NR,
- Figure 3 (SEQ ID NO: 24) presents a polypeptide of the invention of 151 amino acids, designated hereafter as antigen 729S,

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- Figure 4 (SEQ ID NO: 33) corresponds to the nucleotide sequence of the clone DG729S which codes for the polypeptide of figure 3 (EcoRI linkers in bold type),

- Figure 5 presents the polypeptide sequences (SEQ ID NOS: 23 and 26-28) of the antigens LSA-Ter, 729S-NRI, 729S-NRII, 729S-Rep,

- Figure 6 (SEQ ID NO: 34) presents the 5' end of the nucleotide sequence of the LSA gene,

- [Figure 7] Figures 7A-7C (SEQ ID NOS: 35-37) presents the coding sequence of the 5' end of the LSA gene and the corresponding polypeptide sequence,

- Figure 8 (SEQ ID NO: 38) describes the 3' end of the LSA gene,

- [Figure 9] Figures 9A-9D (SEQ ID NOS: 39-42) gives the sequence of the 3' end of the LSA gene as well as the corresponding polypeptide sequence,

- [Figure 10] Figures 10A-10D (SEQ ID NOS: 43-46) repeats the sequences given in [Figure 9] Figures 9A-9D, up to the termination codon stop and the terminal amino acid.

Thus, the present invention relates to any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the amino acid sequence shown in [Figure 9] Figures 9A-9D or Figures 10A-10D, and corresponds to the 3' end of the LSA gene.

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More particularly, the subject of the invention is any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the sequence of the last 279 amino acids shown in Figures 10A-10D, this amino acid sequence being optionally preceded by all or part of one or more of the sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula:

Page 5, Paragraph Beginning at Line 6

Consequently, the invention relates more particularly to any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and more particularly bearing all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the following amino acid sequence (SEQ ID NO: 19):

RKADTKKNLERKKEHGDILAEDLYGRLEIPAIELPS

ENERGYYIPHQSSLPQDNRGNSRDSKEISIIEKTNR

ESITTNVEGRRDIHKGHLEEKDGSIKPEQKEDKS

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this amino acid sequence being optionally preceded by all or part of one or more sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula:

Page 6, Paragraph Beginning at Line 16

The invention relates more particularly to any polypeptide characterized by all or part of the following amino acid sequence (SEQ ID NO: 20):

LQEQQRDLEQRKADTKKNLERKKEHGDILAEDLYGRLEIPAIELPSENERGYY

IPHQSSLPQDNRGNSRDSKEISIIEKTNRESITTNVEGRRDIHKGHLEEKKDG

SIKPEQKEDKS

A preferred polypeptide of the invention is represented by all or part of the following amino acid sequence (SEQ ID NO: 21):

DTKKNLERKKEHGDILAEDLYGRLEIP

(this polypeptide being designated hereafter by the expression LSA-NR (LSA-non-repeated), or also by any sequence derived from the preceding sequence and modified by the substitution of maximally 40% of the amino acids while retaining its physiological activity such as the induction of a response of the T lyphocytes, in particular the cytotoxic T lymphocytes.

Another particularly preferred polypeptide of the invention is characterized by all or part of the following amino acid sequence (SEQ ID NO: 22):

ERRAKEKLQEQQRDLEQRKADTKK

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(this polypeptide being designated hereafter by the expression LSA-J, or LSA-junction, since it overlaps the repetitive part and the non-repetitive part of the molecule shown in Figure 1).

Page 7, Paragraph Beginning at Line 1

Another preferred peptide, designated LSA-TER, is the following (SEQ ID NO: 23):

NSRDSKEISIIEKTNRESITTNVEGRRDIHK

These last three polypeptides are more particularly useful on account of the amphipaticity which characterizes them, and because of their three-dimensional conformation according to the predictions made by the procedure of Chou and Fassmann.

The subject of the invention is also any molecule or polypeptide composition bearing at least one peptide sequence bearing all or part of one or more epitope(s) characteristic of a protein produced at the sporozoite, hepatic and blood (erythrocytic) stages of P. falciparum, and more particularly bearing one or more T epitopes, characterized in that this peptide sequence is represented by all or part of the following amino acid sequence (SEQ ID NO: 24):

RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHNVEEKVE
ESVEENDEESVEENVEENVEENDDGSVASSVEESIASSVDESIDSSIEENVAP
TVEEIVAPTVEEIVAPSVVEKCAPSVEESVAPSVEESVAEMLKER

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shown in Figure 3 and designated hereafter as the polypeptide 729S.

More particularly, the subject of the invention is the amino acid sequence derived from the preceding sequence and characterized by all or part of the following amino acid sequence (SEQ ID NO: 25):

RDELFNELLNSVDVNGEVKENILEESQVNDDIFNSLVKSVQQEQQHN

According to another advantageous embodiment of the invention, sequences of interest derived from the amino acid sequence of the polypeptide 729S are the following (SEQ ID NOS: 26-28):

- DELFNELLNSVDVNGEVKENILEESQ,
- LEESQVNDDIFSNSLVKSVQQEQQHNV,
- VEKCAPSVEESVAPSVEESVAEMLKER.

Page 8, Paragraph Beginning at Line 1

The subject of the invention is also any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, characterized in that this peptide sequence is represented by all or part of the amino acid sequence shown in [Figure 7] Figures 7A-7C.

Consequently, the subject of the invention is more particularly any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one

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or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and bearing more particularly all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence is represented by all or part of the sequence of the first 153 amino acids shown in [Figure 7] Figures 7A-7C, this amino acid sequence being optionally followed by all or part of one or more sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula:

Page 9, Paragraph Beginning at Line 6 to Page 10

The invention also relates to any molecule or polypeptide composition comprising at least one peptide sequence bearing all or part of one or more epitopes characteristic of a protein produced in the hepatocytes infected by P. falciparum, and bearing more particularly all or part of one or more T epitope(s) of the proteins produced at the hepatic stage of P. falciparum, characterized in that this peptide sequence comprises successively:

- all or part of the sequence of the first 153 amino acids shown in [Figure 7] Figures 7A-7C,

- optionally, all or part of one or more of the sequences of 17 amino acids (SEQ ID NOS: 2-18) of formula:

$X_1DLEQX_2RX_3AKEKLQX_4QQ$

$QX_1DLEQX_2RX_3AKEKLQX_4Q$

$QQX_1DLEQX_2RX_3AKEKLQX_4$

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$X_4QQX_1DLEQX_2RX_3AKEKLQ$

$QX_4QQX_1DLEQX_2RX_3AKEKL$

$LQX_4QQX_1DLEQX_2RX_3AKEK$

$KLQX_4QQX_1DLEQX_2RX_3AKE$

$EKLQX_4QQX_1DLEQX_2RX_3AK$

$KEKLQX_4QQX_1DLEQX_2RX_3A$

$AKEKLQX_4QQX_1DLEQX_2RX_3$

$X_3AKEKLQX_4QQX_1DLEQX_2R$

$RX_3AKEKLQX_4QQX_1DLEQX_2$

$X_2RX_3AKEKLQX_4QQX_1DLEQ$

$QX_2RX_3AKEKLQX_4QQX_1DLE$

$EQX_2RX_3AKEKLQX_4QQX_1DL$

$LEQX_2RX_3AKEKLQX_4QQX_1D$

$DLEQX_2RX_3AKEKLQX_4QQX_1$

in which:

° X_1 is "Ser" or "Arg",

° X_2 is "Glu" or "Asp"

° X_3 is "Arg" or "Leu"

° X_4 is "Glu" or "Gly"

- and all or part of the last 279 amino acids shown in [Figure 10] Figures 10A-10D.

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Page 12, Paragraph Beginning at Line 1

The invention also relates to any sequence of nucleotides which codes for a polypeptide identical with, or one similar from the point of view of both structure and antigenic properties to, those of the invention, this sequence being capable of hybridizing with all or part of the nucleotide sequence defined by the nucleotides situated at the positions 597 to 949 of figure 2, or with all or part of the nucleotide sequence of Figure 4 or the sequences complementary to these latter, under the following conditions:

- pre-treatment (pre-hybridization) of the nitrocellulose filter supporting the nucleic acid fragment to be tested with hybridization buffer (composed of 6 SSC, 5x Denhardt's, 0.5% SDS, 100 μg/l denatured, sonicated salmon sperm DNA) this operation being carried out at 65°C for 1 hour;
- replacement of the hybridization buffer in contact with the support to which the nucleic acid fragment is now bound by hybridization buffer of the same composition and addition of the above-mentioned sequence shown in Figure 2 (SEQ ID NO: 32) or Figure 4 (SEQ ID NO: 33) as probe, in particular radioactively labelled, and denatured beforehand;
- incubation of the said nucleic acid fragment bound to the support in this incubation buffer with the above-mentioned sequence shown in Figure 2 (SEQ ID NO: 32) or Figure 4 (SEQ ID NO: 33) at 65°C for a period of about 1 hour;
- the removal of the buffer containing the probe not bound by two successive washings of 30 minutes each with a buffer solution composed of 2 x SSC and 0.5% SDS at 65°C.

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Page 14, Paragraph Beginning at Line 24

As examples of DNA or RNA primers according to the invention, mention should be made of the following sequences (SEQ ID NOS: 29 and 30):

3'→5: TTTCGCTAGATCTTGTT & TCTAAATAGAAGAAA.

Page 19, Paragraph Beginning at Line 10

In fact, as will be described more particularly with the aid of examples of molecules according to the invention in the detailed description which follows, the molecules according to the invention which contain all or part of the amino acid sequence comprised between the positions 200 and 316 shown in Figure 1 (SEQ ID NO: 31), react specifically with the antibodies or the lymphocytes directed against the B and/or T epitopes of the antigens produced at the hepatic stage of P. falciparum, but not with the antibodies directed against other antigens produced by P. falciparum or against antigens produced by other species of Plasmodium.

Page 19, Paragraph Beginning at Line 24 to Page 20

These molecules according to the invention comprising all or part of the peptide sequence shown in Figure 3 (SEQ ID NO: 24) are not recognized by the former antibodies which react specifically with all or part of the polypeptide defined by the amino acids situated at the positions 200 to 316 in Figure 1.

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On the other hand, the polypeptides corresponding to all or part of the peptide sequence shown in figure 3 (SEQ ID NO: 24) are recognized by antibodies which react specifically with antigens localized on the surface of sporozoites (derived from different strains of P. falciparum) as well as with antigens of the hepatic schizonts and the blood schizonts, and finally with the surface of the sporozoites of P. yoelii but not of P. berghei.

It should also be emphasized that the antibodies which recognize specifically the polypeptides corresponding to all or part of the peptide sequence shown in Figure 3 (SEQ ID NO: 24) are capable of blocking completely the entry of the sporozoites of P. yoelii into hepatic cells of rodents in vitro, unlike the antibodies directed against the circumsporozoite protein of P. yoelii and of P. falciparum.

Page 22, Paragraph Beginning at Line 22

As examples of nucleotide probes of the invention, mention should be made of the following sequences:

3'→5' : TTTCGCTAGCTCTTGTT & TCTAAATAGAAGAAA--.

Page 29, Paragraph Beginning at Line 4

The insert of 951 base pairs was purified and recloned in the bacteriophage M13 mp19. The DNA sequence and the genomic organization of the LSA gene were then determined. Figure 1 (SEQ ID NO: 31) shows that the clone contains a sequence of 209

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amino acids at the 5' end corresponding to a series of 12 repeats of 17 amino acids, similar to that described in the article by Guérin-Marchand et al. (Nature, mentioned above) and then contains a set of 106 amino acids, the structure of which is not repetitive.

As can be seen in Figure 1 (SEQ ID NO: 31), the motif of 17 amino acids is in two repeats (cf. motif corresponding to the positions 35 to 51, and that corresponding to the positions 137 to 153 of figure 1) identical with that described in the article by Guérin-Marchand et al. and the other repeats exhibit a substitution of a leucine by an arginine (cf. positions 8, 59, 76, 110, 127, 161, 178 and 195 of Figure 1) (SEQ ID NO: 31), a substitution of a glutamic acid by an aspartic acid (cf. positions 23 and 91 of Figure 1 (SEQ ID NO: 31)) as well as a substitution of a serine by an arginine (cf. position 205 of Figure 1 (SEQ ID NO: 31)).

Between the last page of the Disclosure (page 35) and the first page of the Claims, insert the attached paper copy of the "Sequence Listing".